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TITLE: Promoter-Based Theranostics for Prostate Cancer

PRINCIPAL INVESTIGATOR: Martin Pomper

CONTRACTING ORGANIZATION: Johns Hopkins University
Baltimore, MD 21218

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13. SUPPLEMENTARY NOTES						
14. ABSTRACT The most significant finding of this reporting period has been the demonstration that the clones we generated were indeed capable of concentrating [²¹¹ At]FIAU and that the reporter-containing cells were susceptible to treatment with this alpha-particle emitter.						
15. SUBJECT TERMS molecular imaging; PEG-Prom; AEG-Prom; FIAU; alpha-particle; β-hCG; prostate cancer; HSV1-tk; 1-PEI; nanoparticle						
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Table of Contents

	<u>Page</u>
1. Introduction.....	4
2. Keywords.....	4
3. Accomplishments.....	4
4. Impact.....	6
5. Changes/Problems.....	6
6. Products.....	6
7. Participants & Other Collaborating Organizations.....	6
8. Special Reporting Requirements.....	n/a
9. Appendices.....	n/a

1. INTRODUCTION: This project leverages cancer-specific promoters for molecular-genetic endoradiotherapy and in the development of a universal blood test for cancer. The idea is to have the progression-elevated gene 3 promoter (PEG-Prom) and/or the astrocyte-elevated gene 1 promoter (AEG-Prom) serve to drive, in a cancer-specific manner, production of a thymidine kinase that can phosphorylate radiotherapeutic nucleosides, as well as drive β -hCG, which can be detected using a commercial urine pregnancy test. The technology is based on our previously reported promoter-based cancer-specific imaging. The innovations of the project lie in its simplicity, use of non-viral vectors, systemic delivery and that it is a generalizable, platform technology.

2. KEYWORDS: molecular imaging; PEG-Prom; AEG-Prom; FIAU; alpha-particle; β -hCG; prostate cancer; HSV1-tk; I-PEI; nanoparticle

3. ACCOMPLISHMENTS:

- **What were the major goals of the project?**
 - Cloning strategy for PEG- β -hCG and AEG- β -hCG and AEG-HSV1-tk plasmids (completed 2/15)
 - Generation of PEG- β -hCG and AEG-HSV1-tk plasmids (completed 5/15)
 - Sensitivity testing of β -hCG expression plasmids (completed 7/15)
 - *In vitro* uptake and *in vivo* evaluation of radiolabeled FIAU of HSV1-tk expression vectors (75% complete)
- **What was accomplished under these goals?**

We have successfully generated expression vectors for PEG-Prom driven human chorionic gonadotropin β -chain (β -hCG). When we transfected PC3 and LNCaP prostate cancer cells, we were able to detect human hCG in the culture media using the β -hCG ELISA assay, indicating that the

cancer-specific progression elevated gene 3 promoter (PEG-Prom) was capable of expressing soluble and secreted β -hCG in prostate cancer cell lines (**Fig. 1**). In order to test this *in vivo*, we have developed a mouse model of human metastatic prostate cancer that develops tumors within liver, kidney, and bone after intravenously injecting one million PC3/ML cells tagged with firefly luciferase (fLuc) to NOD/SCID/IL2ry^{null} (NSG) mice (**Fig. 2**). This model starts to develop detectable metastatic lesions three weeks after the injection and dies at around the 6th week due to metastatic disease. The tumor expresses fLuc for non-invasive tracking of metastatic tumor development. We injected pPEG-hCG vectors formulated with *in vivo* jetPEI for systemic delivery to our metastatic prostatic cancer bearing mice and healthy mice as a control group. Forty eight hours after the injection of the nanoplex, we collected blood

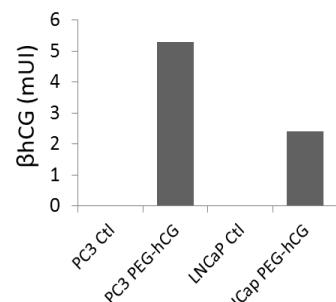


Fig. 1. PEG-Prom driven hCG was successfully expressed and secreted into the media from PC3 and LNCaP cells.

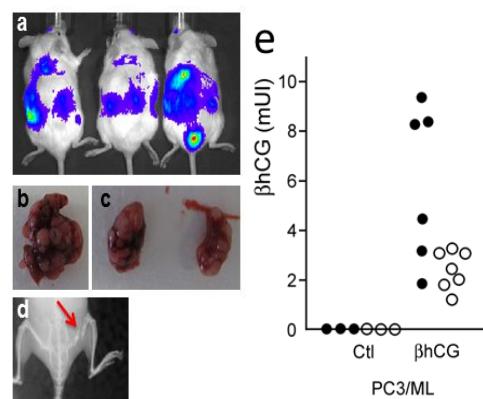


Fig. 2. PEG-Prom driven hCG was successfully expressed and secreted to serum and urine of Experimental Metastatic mouse model of human prostate Cancer. (a) Bioluminescent images of mice with metastatic model. (b) Liver metastasis. (c) Kidney metastasis. (d) Lytic bone metastasis (arrow). (e) Serum (closed circles) and urine (open circles) level of β -hCG. Each circle represents one mouse.

and urine from each mouse and measured the level of human β hCG. We were able to detect soluble β -hCG in both serum and urine of tumor bearing mice whereas there no detectable β -hCG from healthy mice (**Fig. 2e**). We have even shown that we could detect β -hCG from the urine of tumor-bearing mice using a commercial pregnancy test (**Fig. 3**). The model and sensitivity for detection are being tested and optimized, where possible.

We are currently testing our therapeutic approach using PEG-Prom-driven HSV1-tk as a therapeutic gene and [^{211}At]FIAU as a source of radiotherapy (alpha-particles) for the second part of the proposal.

We successfully created the therapeutic vector harboring the PEG-Prom and HSV1-tk. When we transfected the PC3/ML cell line with the vector, the transfected cells showed specific uptake of its therapeutic substrate [^{211}At]FIAU (**Fig. 4A**). Cells expressing PEG-Prom-driven HSV1-tk also exhibited dose-dependent cell kill by [^{211}At]FIAU (**Fig. 4B**). Encouraged by these *in vitro* results, we are currently performing the corresponding *in vivo* therapeutic study using the metastatic PC model described in **Fig. 2**. In addition, we have initiated long-term *in vivo* toxicity studies of [^{211}At]FIAU in varying doses.



Fig. 3. Detection of PEG-Prom driven (cancer specific) production of β hCG using a commercial urine pregnancy test. Note faint line of detection on right. This indicates feasibility of universal cancer detection using a simple and available kit.

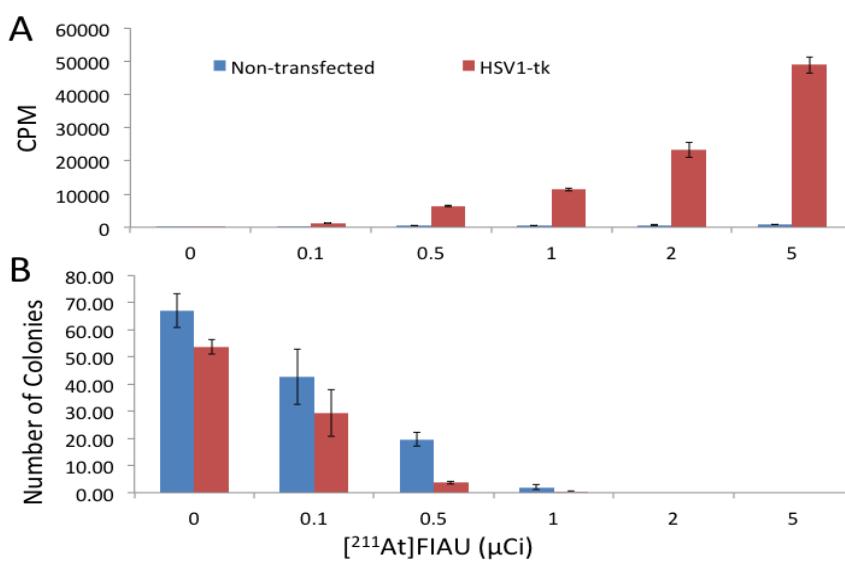


Fig. 4. (A) *In vitro* radio-uptake assay. Only PC3/ML cells transfected expressing PEG-Prom-driven HSV1-tk showed dose dependent uptake of [^{211}At]FIAU. (B) Clonogenic survival assay of PC3/ML cells treated with [^{211}At]FIAU. Cells expressing PEG-Prom-driven HSV1-tk were susceptible to the treatment. CPM = counts per minute.

- **What opportunities for training and professional development has the project provided?**
 - Although not necessarily intended for this purpose, the project will prove instrumental in the professional development of Dr. Il Minn, currently an instructor in radiology, who will be proposed soon for assistant professor. Furthermore, Dr. Minn has engaged two technicians whom he has trained in the cloning and other studies necessary to undertake this work.
- **How were the results disseminated to communities of interest?**
 - Nothing to report at the present time.

- **What do you plan to do during the next reporting period to accomplish the goals?**
 - During the requested extension we intend to finish the *in vivo* studies and, if possible, optimize the proposed urine test which is working but may not be sufficiently sensitive to compete with other ways to detect cancer in body fluids, e.g., naked DNA or circulating tumor cells. We would like a more quantitative sense of the sensitivity of the device.
- 4. **IMPACT:**
 - **What was the impact on the development of the principal discipline(s) of the project?**
 - This is the first project to use β-hCG to detect cancer universally. It is also the first to use, in real living systems, an alpha-particle-emitting, targeted radiotherapeutic agent, namely, [²¹¹At]FAAU.
 - **What was the impact on other disciplines?**
 - Nothing to report at this time as we have not yet published.
 - **What was the impact on technology transfer?**
 - Nothing to report at this time.
 - **What was the impact on society beyond science and technology?**
 - Nothing to report at this time.
- 5. **CHANGES/PROBLEMS:** Nothing to report
- 6. **PRODUCTS:** Nothing to report – These items will be reported once finished with the project and publications are submitted.
- 7. **PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS**
 - **What individuals have worked on the project?**
 - No change
 - **Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?**
 - Marty Pomper

Ended

Title: PSMA-Based Cancer Imaging Agents

Time Commitments: 0.24 calendar months

Supporting Agency: NCI, R01CA134675 (NCE)

Grants Contact: Barbara Croft (301) 496-9531 E-Mail: croftb@mail.nih.gov

PI: Martin Pomper

Performance Period: 4/1/2009-2/28/2015

Level of Funding: \$159,199

Description of Goals: Prostate cancer (PCa) is the leading cancer in the U.S. population and the second leading cause of cancer death in men (16). Therapy for locally advanced disease remains contentious and an increasing number of disparate options are available. Perhaps the most pressing issue in PCa management is the need to predict, at the time of diagnosis, which tumors will remain indolent and which will progress rapidly. The ability to fulfill that goal would eliminate the prostate-specific antigen (PSA)-mediated over detection and overtreatment of clinically insignificant disease.

Aim #1: Synthesis and evaluation of a series of PET imaging agents for PSMA.

Aim #2: Synthetic optimization of the best compounds of Aim 1 en route to GMP and/or facilitated use.

Aim #3: Synthesis and evaluation of a series of homo- and heterodimeric imaging agents for PSMA.

Title: BETR Therapy of Herpesvirus-associated Tumors

Time Commitments: 1.09 calendar months

Supporting Agency: NCI, NIH R01CA138636

Grants Contact: Jason Gill (301) 496-7240 E-Mail:gilljas@mail.nih.gov

PI: Martin Pomper

Performance Period: 04/01/10-02/28/15

Level of Funding: \$322,099

Description of Goals: The purpose is to treat gammaherpesvirus-associated tumors with [131]FIAU in human subjects

Aim #1: To perform a first-in-man, FIAU-PET image-guided, BETR study in patients with EBV-associated malignancies.

Aim #2: To assess parameters that will aid in the optimization of therapy.

Title: TK-based Infection Imaging

Time Commitments: 0.24 calendar months

Supporting Agency: NIH, NIBIB R01EB009367 (NCE)

Grants Contact: Florence Turska (301) 496-9314 E-Mail:ft7p@nih.gov

PI: Martin Pomper

Performance Period: 05/15/10-04/30/15

Level of Funding: \$267,740

Description of Goals: The goal is to study further musculoskeletal infection, comparing a newly developed method in infection imaging to the current clinical standard of tagged white blood cell (WBC) and attempting to determine the sensitivity and specificity of our technique.

Aim #1: Estimate the sensitivity and specificity of FIAU-PET in detecting orthopedic infection.

Aim #2: To extend the FIAU imaging technique to pulmonary infection.

Aim #3: To transition from [124I]FIAU to [18F]FIAU for imaging bacterial infection.

Title: Precision Measurement in Rheumatoid Arthritis

Time commitments: 0.09 calendar months

Supporting Agency: Sibley Hospital 90048894 (NCE)

Grants Contact: Robert L. Sloan, President and CEO; 5255 Loughboro Rd, N.W., Washington DC 20016; 202-537-4680

PI: Rosen

Role: Co-Investigator

Performance Period: 11/1/2011-10/31/2014

Level of Funding: \$600,035

Description of Goals: The long term goal of this aim is to improve the utility of MR imaging in evaluation of RA

Aim 1: A graded approach, extending from basic studies to those with an obvious pathway to clinical translation by providing the following specific aims, which focus on molecular imaging.

Aim 2: A graded approach, extending from basic studies to those with an obvious pathway to clinical translation by providing the following specific aims, which focus on high-field magnetic resonance (MR) (Aim 2) imaging.

Title: Molecular Imaging for Macrophage-Associated Pulmonary Inflammation

Time commitments: 0.36 calendar months

Supporting Agency: NIH/NHLBI 1R01HL116316

Grants Contact: Kimberly Stanton, (301) 435-0519, E-Mail stantonk@nhlbi.nih.gov

PI: Sanjay Jain

Performance Period 9/25/2012- 6/30/2015

Level of Funding: \$238,000

Role: Co-Investigator

Description of Goals: The overall goal is to have a fully validated probe ready for human administration and to file a FDA Investigational New Drug (IND) application at the end of the funding period.

Aim 1: To evaluate [125/I]DPA-713-SPECT/ PET as a biomarker for serial monitoring of macrophage-associated pulmonary inflammation.

Aim 2: To perform cGMP synthesis and toxicology studies for iodo-DPA-713.

Aim 3: To quantify and correlate lesion-specific, multi-modality image parameters across different time-points using in-house computer-assisted image analysis tools.

Title: Extrathalamic nAChR-PET for Imaging Neurodegeneration

Time Commitments: 0.46 calendar months

Supporting Agency: NIH R33AG037298

Grants Contact: Jessica Perez, 301-496-1472, E-Mail: perezj@nia.nih.gov

PI: Andrew Hortsch

Role: Co-Investigator

Performance Period: 03/1/2011-8/31/2015

Level of Funding: \$249,274

Description of Goals: The goal is to develop a new nicotinic receptor-based PET agent that enables imaging of extrathalamic sites.

Aim 1, R21. To develop a method of synthesis of sufficient quantities (100-300 mg) of precursor (-)JHU87571 for radiolabeling of [18F]XTRA for 100 radiosyntheses.

Aim 2, R21. To evaluate [18F]XTRA in mice. (a) To confirm that *in vivo* [18F]XTRA binds at nAChR selectively and specifically. (b) To show that the radioactive metabolites are not present in the mouse brain. (c) To carry out radiation dosimetry studies in mice for an eIND application.

Aim 3, R21. To characterize [18F]XTRA in baboon PET studies. (a) To confirm that the high nAChR binding potentials in cortex, hippocampus and putamen ($BP \geq 1.1$) and optimally rapid brain kinetics were not unique to the single experiment of the Preliminary studies.

Title: Multi-Color Exchange Transfer Imaging of Drug Delivery Nanocarriers

Time Commitments: 0.09 calendar months

Supporting Agency: NIH R01EB01531

Grants Contact: Guoying Liu, 301-594-5220, E-Mail: liug@mail.nih.gov

PI: Michael McMahon

Role: Co-Investigator

Performance Period: 8/1/2011-6/30/2015

Level of Funding: \$439,205

Description of Goals: This proposal is focused on the production of carriers for cervical tumor drugs which are labeled with DIACEST contrast agents for MRI monitoring.

Aim #1: To design a library of peptide-based DIACEST contrast agents suitable for incorporation into biodegradable particles

Aim #2: To design CEST drug carriers optimized for systemic nanoparticle-based chemotherapy

Aim #3: (A) To design CEST drug carriers optimized for local nanoparticle-based chemotherapy. (B) To test imaging after local and systemic administration.

New

Title: PSMA Directed Imaging of Prostate Cancer Focus on Androgen Receptor Dynamics

Time Commitments: 1.35

Supporting Agency: NIH/NCI U01CA183031

Grants Contacts: Yantian Zhang; Program Official; 240-276-5980; Yantian.zhang@nih.gov

PIs: Pomper/Deweese

Performance Period: 11/01/2014-10/31/2016

Level of Funding: \$496,642

Description of Goals: The overall goal is to validate at least two positron-emitting, PSMA-targeted imaging agents clinically so that they can be used to full advantage in supporting existing and emerging therapies for a spectrum of patients suffering from PCa.

Aim 1. To image treatment-naïve patients with localized-locally advanced primary PCa using DCFBC-PET/magnetic resonance imaging, and correlate signal with that on MR concurrently obtained, as well as with tumor grade, PSMA expression and androgen receptor (AR) signaling before and after two months of neoadjuvant androgen deprivation (ADT).

Aim 2. To image patients with CRPC using DCFBC-PET/MR and correlate findings with bone and soft tissue biopsy.

Aim 3. To image patients with CRPC with DCFBC-PET/MR and correlate with standard 99mTc-based bone scan to guide stereotactic body radiation treatment (SBRT) in patients with oligometastatic disease.

Aim 4. Imaging CRPC with the second-generation, PSMA-targeted PET agent, [18F]DCFPyL.

Title: High-Specificity Imaging Agents for Aggressive Prostate Cancer

Time commitments: 1.35 calendar months

Supporting Agency: NIH/NCI (Renewal) R01CA134675

Grants Contact: Leota Hall; Program Official; 240-276-6449; halle@gmail.nih.gov

PI: Pomper

Performance Period: 12/1/2014-11/30/2019

Level of Funding: \$443,885

Description of Goals: The goals of this project are to leverage existing but untested agents and to develop new agents for imaging PC, with a focus on aggressive, localized disease.

Aim 1: Imaging of patients with biopsy-proved primary PC with DCFPyL-PET with subsequent correlation of PET signal with histopathology at prostatectomy for PSMA expression, Gleason score and other markers

Aim 2: Synthesis of select PSMA-targeted imaging agents that (a) encompass a new scaffold to engender superior affinity and pharmacokinetics; (b) are hetero-bivalent (HtBv), homing to a rationally chosen co-target (in addition to PSMA); or, (3) enable detection with MR through signal amplification

Aim 3: Development and testing of new agents for imaging the PC microenvironment

Title: Direct Test for Neuroinflammation with [11C]DPA-713-PET Scanning

Time commitments: 1.20 calendar months

Supporting Agency: DoD W81XWH-14-1-0620

Grants Contact: Kathy Robinson, GWIRP Grants Officer; 820 Chandler St, Fort Detrick MD 21702

PI: Pomper

Period of Performance: 07/01/2014-06/30/2019

Level of Funding: \$389,978

Description of Goals: This project concerns measuring two key neurological aspects of Gulf War Illness (GWI), namely, neuroinflammation and dysregulation of muscarinic cholinergic transmission.

Aim 1. To assess the degree of microglial activation in the brains of former Gulf War veterans who suffer from GWI through [11C]DPA-713 PET.

Title: Bipolar Androgen Therapy: Breaking out of the Chrysalis of Chronic Androgen Deprivation Therapy in Men with Late-Stage Castrate Resistant Prostate Cancer

Time commitments: 0.12 calendar months

Supporting Agency: CDMRP

Grants Contact: TBD

PI: Denmeade

Co-Investigator: Pomper

Performance Period: 09/1/2014-08/31/2017

Level of Funding: \$1,669,328

Aim 1: The major objective is to demonstrate the superiority of BAT vs. Enza in asymptomatic men with metastatic CRPC progressing after ADT and Abi, by performing a multi-institutional, open-label, randomized study, using radiographic progression-free survival (rPFS) as the primary endpoint.

Aim 2: Evaluate the effect of BAT on the uptake of FDHT and PSMA inhibitor-based PET agents in metastatic sites.

Aim 3: Evaluate regulation of AR splice variants in circulating tumor cells (CTCs) in response to therapy.

Aim 4. Analyze circulating tumor DNA to determine the effect of individual therapies on emergence of AR mutations.

- IL Minn

Ended

Title: Promoter-driven Molecular Radiotherapy for Prostate Cancer
Time Commitments: 1.80 calendar months
Supporting Agency: Prostate Cancer Foundation
Grants Contact: Howard Soule, Chief Science Officer
PI: Pomper
Role: Co-Investigator
Performance Period: 10/15/12-10/15/2014
Level of Funding: \$500,000
Description of Goals: We propose a radical, new method for treating both primary and metastatic prostate cancer (PCa).
Aim 1. Optimize the nanoparticle delivery system with respect to the key features of toxicity, long circulation stability and high in vivo transfection efficiency.
Aim 2. Construct a PEG-Prom-driven gene that will place (strept)avidin on the surface of PCa cells.
Aim 3. To use our existing PEG-Prom-driven HSV1-TK system to enable sequestration of an α -particle emitter, [^{211}At]FAAU, specifically within PCa to afford selective tumor cell kill.

New

Title: High-Specificity Imaging Agents for Aggressive Prostate Cancer
Time commitments: 1.80 calendar months
Supporting Agency: NIH/NCI (Renewal) R01CA134675
Grants Contact: Leota Hall; Program Official; 240-276-6449; halle@gmail.nih.gov
PI: Pomper
Role: Co-Investigator
Performance Period: 12/1/2014-11/30/2019
Level of Funding: \$443,885
Description of Goals: The goals of this project are to leverage existing but untested agents and to develop new agents for imaging PC, with a focus on aggressive, localized disease.
Aim 1: Imaging of patients with biopsy-proved primary PC with DCFPyL-PET with subsequent correlation of PET signal with histopathology at prostatectomy for PSMA expression, Gleason score and other markers
Aim 2: Synthesis of select PSMA-targeted imaging agents that (a) encompass a new scaffold to engender superior affinity and pharmacokinetics; (b) are hetero-bivalent (HtBv), homing to a rationally chosen co-target (in addition to PSMA); or, (3) enable detection with MR through signal amplification
Aim 3: Development and testing of new agents for imaging the PC microenvironment

Title: PSMA Directed Imaging of Prostate Cancer Focus on Androgen Receptor Dynamics
Time Commitments: 2.4
Supporting Agency: NIH/NCI U01CA183031

Grants Contacts: Yantian Zhang; Program Official: 240-276-5980;
Yantian.zhang@nih.gov
PIs: Pomper/Deweese
Performance Period: 11/01/2014-10/31/2016
Level of Funding: \$496,642
Description of Goals: The overall goal is to validate at least two positron-emitting, PSMA-targeted imaging agents clinically so that they can be used to full advantage in supporting existing and emerging therapies for a spectrum of patients suffering from PCa.
Aim 1. To image treatment-naïve patients with localized-locally advanced primary PCa using DCFBC-PET/magnetic resonance imaging, and correlate signal with that on MR concurrently obtained, as well as with tumor grade, PSMA expression and androgen receptor (AR) signaling before and after two months of neoadjuvant androgen deprivation (ADT).
Aim 2. To image patients with CRPC using DCFBC-PET/MR and correlate findings with bone and soft tissue biopsy.
Aim 3. To image patients with CRPC with DCFBC-PET/MR and correlate with standard 99mTc-based bone scan to guide stereotactic body radiation treatment (SBRT) in patients with oligometastatic disease.
Aim 4. Imaging CRPC with the second-generation, PSMA-targeted PET agent, [18F]DCFPyL.

Title: Specific Molecular Imaging Agents for Clear Cell Renal Cell Carcinoma Diagnosis
Time Commitments: 0.6
Supporting Agency: NIH/NCI R03CA197470
Grants Contacts: Houston Baker, PO, 240-276-5908, bakerhou@mail.nih.gov; Jacquelyn Boudjeda, GMS, 240-276-6312, boudjedaj@mail.nih.gov
PIs: Yang
Performance Period: 07/01/15-06/30/17
Level of Funding: \$50,000
Description of Goals: The primary goal is to develop small molecule imaging agents specifically targeting clear cell renal cell carcinoma using carbonic anhydrase IX as a target.
Title: Shape control and transport properties of DNA-templated micells
Time Commitments: 3.6
Supporting Agency: NIH/NIBIB R01EB018358
Grants Contacts: Jessica Tucker, PO, 301-451-4778, tuckerjm@mail.nih.gov; Ruthann Rand, GMS, 301-496-8521, randrudy@mail.nih.gov
PIs: Mao
Performance Period: 07/01/15-06/30/19
Level of Funding: \$125,000
Description of Goals: The ultimate goal is to develop better nanoparticles for delivery gene in vivo.

- **What other organizations were involved as partners?**

- Nothing to report